Protective effects of apigenin against methyl methanesulfonate induced hsp70 expression in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ)Bg*^{9}

Sir,

Hsp70 are a family of predominantly expressed heat shock proteins of ubiquitously expressed heat shock proteins.[1] In recent years, hsp70 has been considered to be one of the candidate genes for predicting cytotoxicity against environmental chemicals.[2]Methylmethanesulfonate (MMS) is not only classified as a mutagen, but also as a carcinogenic agent. Exposure to MMS appears to be limited to laboratory research persons.[3]

Apigenin is one of the several active ingredients found naturally in many fruits and vegetables. It is recognized in traditional or alternative medicine for its pharmacological activity.[4] Now-a-days the use of animals in toxicological/pharmacological research and testing has become an important issue for both science and ethics. As a result, the emphasis has been given to the use of alternative to mammals in testing, research, and education. The European Centre for the Validation of Alternative Methods (EVCAM) has recommended the use of *Drosophila* as an alternative model for scientific studies.[5] In our present study, an attempt has been made to validate this model for the evaluation of the chemotherapeutic/natural plant products for their protective action.

A transgenic *Drosophila melanogaster* line expressing bacterial β-galactosidase as a response to stress was used in this study.[6] The flies and larvae were cultured on standard *Drosophila* food containing agar, cornmeal, sugar, and yeast at 24°C. MMS at 0.5 and 1.0 μl/ml of food concentration alone and along with 0.1, 0.5, and 1.0 μl/ml of apigenin were established. The third instar larvae were allowed to feed on them for different time intervals (12, 24, and 48 h). For quantifying the β-galactosidase activity, the method as described by Nazir *et al*. was followed.[6] The extent of tissue damage in larvae exposed to different concentrations of MMS alone and along with apigenin was assayed by a dye exclusion test.[6] Statistical analysis was carried out by Student's '*t*'-test. $P<0.05$ was considered statistically significant.

The results of this study reveal that the exposure of 0.5 and 1.0 μl/ml of MMS to the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg*^{9} for the duration of 12, 24, and 48 h showed an increase in the activity of

Table 1: β-Galactosidase activity measured in transgenic *Drosophila melanogaster (hsp70-lacZ)Bg*^{9} third instar larvae exposed to different concentrations of methyl methanesulfonate for various time intervals

Treatments	OD (Mean ± SE) after 12 h	OD (Mean ± SE) after 24 h	OD (Mean ± SE) after 48 h
MMS (μl/ml)			
0.5	0.265 ± 0.012[a]	0.282 ± 0.014[a]	0.294 ± 0.015[a]
1.0	0.313 ± 0.023[a]	0.324 ± 0.028[a]	0.332 ± 0.033[a]
MMS (μl/ml) + Apigenin (μl/ml)			
0.5 + 0.1	0.243 ± 0.011[ab]	0.262 ± 0.012[ab]	0.275 ± 0.13[ab]
0.5 + 0.5	0.238 ± 0.009[b]	0.251 ± 0.011[ab]	0.263 ± 0.011[ab]
0.5 + 1.0	0.232 ± 0.009[b]	0.248 ± 0.012[ab]	0.241 ± 0.012[ab]
1 + 0.1	0.189 ± 0.014[ab]	0.301 ± 0.015[ab]	0.314 ± 0.24[ab]
1 + 0.5	0.272 ± 0.013[ab]	0.293 ± 0.014[ab]	0.304 ± 0.22[ab]
1 + 1	0.261 ± 0.010[ab]	0.283 ± 0.013[ab]	0.292 ±0.19[ab]
Apigenin (μl/ml)			
0.1	0.223 ± 0.008	0.223± 0.009	0.234 ± 0.010
0.5	0.220 ± 0.007	0.214± 0.005	0.221 ± 0.008
1.0	0.221 ± 0.007	0.228± 0.008	0.230 ± 0.011
Untreated	0.212 ± 0.006	0.220 ± 0.009	0.218 ± 0.007
DMSO (5 μl/ml)	0.223 ± 0.008	0.222 ± 0.009	0.221 ± 0.009

[a]Significant at $P<0.05$ compared to untreated. [b]Significant at $P<0.05$ compared to MMS treatment

Table 2: Regression analysis for the β-galactosidase activity in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ)Bg⁹ to study the dose* effect of apigenin (i.e. 0.1, 0.50 and 1 μl/ml) on 0.5 and 1.0 ml/ml of MMS for 12, 24 and 48 h of exposure

Treatments MMS	Duration (h)	Regression equation	*r*-value	β-coefficient	SE	*P value*	*F*-value
0.5	12	$Y = 0.24437–0.0123X$	−0.999	−1.0	0.000	<0.004	3346.68
0.5	24	$Y = 0.26144–0.0145X$	−0.926	−0.93	0.004	<0.009	5.98
0.5	48	$Y = 0.28031–0.0383X$	−0.994	−0.99	0.003	<0.006	87.57
1	12	$Y = 0.2986–0.0641X$	−0.987	−0.99	0.007	<0.0146	36.75
1	24	$Y = 0.30313–0.199X$	−1.0	−1.0	0.000	<0.0003	11598.87
1	48	$Y = 0.31651–0.0248X$	−0.999	−1.0	0.467	<0.023	681.28

hsp70 expression [Table 1]. The exposure of third instar larvae of MMS along with the 0.1, 0.5, and 1.0 μl/ml of apigenin for 12 h results in the reduction of the activity of *hsp70* expression [Table 1]. Similar results were obtained for 24 and 48 h of exposure [Table 1]. The exposure of third instar larvae to 1.0 μl/ml of apigenin for 12 h results in the reduction of the expression of *hsp70* activity [Table 2]. Similar results were obtained for the 24 and 48 h of exposure to 1.0 μl of MMS along with the 0.1, 0.5, and 0.1 μl/ml of apigenin [Table 1]. The regression analysis was also performed to study the dose and duration effects for the exposure of third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg⁹* to MMS and apigenin in combination [Table 2]. The exposure of third instar larvae for 12 h to 0.5 μl/ml of MMS along with 0.1, 0.5 and 1.0 μl/ml of apigenin was associated with the β-coefficient of −1.0 ($F = 3346.680$). The reduction in the β-coefficient values was also observed for other durations of exposure and combinations [Table 2]. The reduction in the value of β-coefficient demonstrates the reduction in the β-galactosidase activity. Trypan blue staining was performed to study the tissue damage induced by MMS in the larval tissue exposed to different doses of MMS alone and in combination with apigenin. About 92% of the larvae of untreated were negative to trypan blue staining even after 48 h of the treatment. About 85% of the larvae showed staining in midgut of the larvae exposed to 0.5 and 1.0 μl/ml of MMS to 12 h. For higher duration of exposure, i.e. 24 and 48 h, the damage was observed in the brain ganglia, midgut, salivary glands, malpighian tubules, and the hind gut. The damage was reduced when the apigenin was mixed in the diet. About 43% of larvae show light staining in the midgut exposed to 12 h of duration to 1.0 μl/ml of MMS along with the 1.0 μl/ ml of apigenin. The damage was further reduced 24 and 48 h of exposure. The damage was observed only in the salivary gland, and no damage was observed in midgut, malpighian tubule, and hind gut. The results of this study reveals that the apigenin is potent enough in reducing the toxic effects of MMS in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg⁹*. *Drosophila* may be used as a system for ADR detection and management. The same system may also be used for faster drug development which could be cost efficient. Although the mammalian system may represent more accurate evaluation tools of short terms and safety, they are frequently laborious and costly at early stages of drug discovery and development.[7]

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REFERENCES

1. Tavaria M, Gabriele T, Kola I, Anderson RL. A hitchhiker's guide to the human *hsp70* family. Cell Stress Chap 1996;1:23-8.
2. Mukhopadhyay I, Nazir A, Mahmood K, Saxena DK, Das M, Khanna SK, *et al*. Toxicity of argenone oil: Effect on hsp70 expression and tissue damage in transgenic *Drosophila melanogaster* (hsp70-lacZ)Bg⁹. Cell BiolToxicol 2002;18:1-11.
3. HSDB. Hazardous substances database. National Library of Medicine, 2000.
4. Peterson J, Dwyer J. Flavonoids: Dietary occurrence and biochemical activity. Nutr Res 1998;18:1995-2018.
5. Festing MF, Baumans V, Combes DR, Halder M, Hendricsen FM, Howard BR *et al*. Reducing the use of laboratory animals in biomedical research: Problems and possible solutions. Alt Lab Anim 1998;26:283-301.
6. Nazir A, Mukhopadhyay I, Saxena DK, Siddiqui MS, Chowdhuri DK. Evaluation of toxic potential of captan: Induction of *hsp70* and tissue damage in transgenic *Drosophila melanogaster* (hsp70-lacZ)Bg⁹. J BiochemMolToxicol 2003;17:98-107.
7. Avanesian A, Semmani S, Jafri M. Can *Drosophila melanogaster* represent a model system the detection of reproductive adverse drug reactions? Drug Dis Today 2009;14:761-6.

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